12. Heterozygous mice that were observed up to 9 months of age did not show behavioral abnormalities during normal handling. Proteins from brains of 4-month-old heterozygotes and sex-matched littermates (n = two pairs) were analyzed by protein immunoblot with HF1 and HP1, polyclonal rabbit antisera to human huntingtin directed against epitopes near the COOH-terminus and the NH_2-terminus, respectively (*5*). Neuropathological examination of 4-month-old heterozygotes and sex-matched CD1 littermates (n = four pairs) was carried out on hematoxylin-eosin stained 7-μm serial sections of parafin-embedded whole brain, including the brain stem (*2*). In sections of the cortex, cerebellum, and basal ganglia, the number of abnormal neurons (five fields) was counted, and no significant morphological alterations between wild-type and heterozygous animals were observed. Neuropathological examination of 4-month-old heterozygotes and sex-matched C57BL/6J littermates (n = two pairs) was carried out on similar serial sections, with adjacent sections being stained with hematoxylin-eosin, hematoxylin-eosin-Luxol fast blue, and cresyl violet (Nissl). Blinded examination by three neuropathologists detected no diagnostic abnormality in any region, including the basal ganglia (caudate putamen, globus pallidus, amygdala, substantia nigra reticulata and compacta, and subthalamic nucleus).
13. RNA in situ hybridization of dissected embryos was done as described in R. A. Conlon and B. G. Herrmann, *Methods Enzymol.* **225**, 373 (1993), with the use of the following probes: *Brachyury* (*T*) [B. G. Herrmann, *Development* **113**, 913 (1991)]; *gsc* [M. Blum *et al.*, *Cell* **69**, 1097 (1992)]; *HNF-3β* [A. P. Monaghan, A. P. Kessner, E. Grau, G. Schutz, *Development* **119**, 567 (1993)]; and *Hdh* (from a 1852-bp Pst I fragment representing bases 7819 to 9670 of mouse *Hdh* complementary DNA, GenBank no. L28827).
14. J. Nasir *et al.*, *Cell* **81**, 811 (1995).
15. A. J. Copp, *Trends Genet.* **11**, 87 (1995).
16. P. J. Willems, *Nat. Genet.* **8**, 213 (1994).
17. A. J. M. H. Verkerk *et al.*, *Cell* **65**, 905 (1991); M. Pieretti *et al.*, *ibid.* **66**, 817 (1991); J. S. Sutcliffe *et al.*, *Hum. Mol. Genet.* **1**, 397 (1992).
18. The Dutch-Belgium Fragile X Consortium, *Cell* **78**, 23 (1994).
19. *Gene Targeting: A Practical Approach*, A. L. Joyner, Ed. (Oxford Univ. Press, Oxford, 1993).
20. The authors thank E. Li and M. Kownacka for assistance in culturing ES cells; S. Chui for his expert help with the histology; E. P. Richardson for neuropathological consultation; J. Rossant for marker probes; and N. Rosenthal, C. Neville, and B. Morgan for valuable discussion. Supported by NIH grants NS32765 and NS16367 and by grants from Bristol-Myers Squibb Inc. (J.F.G. and A.J.), the Hereditary Disease Foundation, and the Huntington's Disease Society of America. S.M. and M.D. were supported by fellowships from the Huntington's Disease Society of America and the Hereditary Disease Foundation, respectively. During the course of the work, A.J. was a Medical Research Council of Canada Scientist and International Scholar of the Howard Hughes Medical Institute.

12 April 1995; accepted 22 June 1995

Permeation Selectivity by Competition in a Delayed Rectifier Potassium Channel

Stephen J. Korn and Stephen R. Ikeda

Permeation selectivity was studied in two human potassium channels, Kv2.1 and Kv1.5, expressed in a mouse cell line. With normal concentrations of potassium and sodium, both channels were highly selective for potassium. On removal of potassium, Kv2.1 displayed a large sodium conductance that was inhibited by low concentrations of potassium. The channel showed a competition mechanism of selectivity similar to that of calcium channels. In contrast, Kv1.5 displayed a negligible sodium conductance on removal of potassium. The observation that structurally similar potassium channels show different abilities to conduct sodium provides a basis for understanding the structural determinants of potassium channel selectivity.

Delayed rectifier K^+ channels are exposed to large electrochemical gradients of both Na^+ and K^+. Rapid repolarization of the action potential therefore requires that these channels be highly selective for K^+ over Na^+. K^+ channels contain multiple ion binding sites within the pore that can be occupied simultaneously by more than one ion (*1*, *2*). The observation that K^+ currents are partially blocked by Na^+ suggests that Na^+ can bind within the channel pore (*3*, *4*). However, K^+ channels have almost universally been shown to be impermeable to Na^+ (*3*–*5*). The mechanism that prevents the small Na^+ ion from conducting, while permitting several larger ions (for example, K^+, Rb^+, NH_4^+, and Cs^+) to conduct, has not been elucidated.

L-Type Ca^{2+} channels represent another class of multi-ion channels that displays extremely high ion specificity. However, removal of Ca^{2+} permits Na^+ to permeate, which has led to a model in which competition between Ca^{2+} and Na^+ for intrapore binding sites determines which ion occupies the channel and conducts (*6*).

Recently, neuronal K^+ channels from rat superior cervical ganglia and chick dorsal root ganglia were shown to conduct Na^+ on removal of K^+ (*7*). The selectivity mechanism of these channels appeared similar to that of Ca^{2+} channels. We now show that two structurally similar K^+ channels allow Na^+ to permeate in the absence of K^+. However, whereas Na^+ conductance through one channel (Kv2.1) was substantial, Na^+ conductance through the other (Kv1.5) was negligible.

Mouse L cells were injected with complementary RNA that encoded one of two human K^+ channels, Kv2.1 or Kv1.5 (*8*). One day after injection, membrane currents carried by K^+ or Na^+ through these channels were examined by the whole-cell patch clamp technique (*9*). In solutions that contained high external Na^+ and high internal K^+ concentrations (*10*), both channels displayed typical depolarization-evoked delayed rectifier K^+ currents (Figs. 1A and 2A). With K^+ and Na^+ equilibrium potentials set to −87 and >+125 mV, respectively, currents through Kv2.1 reversed at −80 mV (Fig. 1B), indicating that they were carried almost exclusively by K^+.

In cells containing Kv2.1 channels, elimination of both intracellular and extracellular K^+ resulted in large inward currents during both depolarization and repolarization steps (Fig. 1C). With *N*-methylglucamine (NMG^+) in the pipette solution, no outward current occurred with depolarizations as high as +80 mV (Fig. 1D). With symmetrical intracellular and extracellular Na^+ concentrations and asymmetrical Cl^- concentrations (and no K^+), currents through Kv2.1 reversed at the calculated Na^+ equilibrium potential.

Kv1.5 channels carried a measurable but negligible Na^+ current after removal of intracellular and extracellular K^+ (Fig. 2B). Channels that carried little Na^+ current did, however, carry large inward currents when extracellular Na^+ was replaced by K^+ (Fig. 2B). At equimolar ion concentrations, inward conductance of Na^+ was 0.79 ± 0.20% (mean ± SEM; n = 4) of that of K^+ at −20 mV (potential of peak conductance).

In mixtures of two permeant ions, multi-occupancy channels are predicted to display anomalous mole fraction behavior (*2*, *6*). In this situation, addition of low concentrations of the ion with higher affinity for an intrapore binding site should inhibit current through the channel. As the concentration of the high-affinity ion is increased, conductance will increase as a result of electrostatic repulsion between multiple ions in the pore. Kv2.1 displayed anomalous mole fraction behavior in mixtures of Na^+ and K^+ (Fig. 3). In cells with 140 mM Na^+ outside and 140 mM NMG^+ inside, addition of low concentrations of extracellular K^+ resulted in a concentration-dependent decrease in inward Na^+ current (Fig. 3, A and C). The minimum current, measured at +20 mV, occurred at ~3 mM K^+ (*11*). Further increases in the extracellular K^+ concentration resulted in an increase in current magnitude. At a concentration of 140 mM of exclusively one ion, inward conduc-

S. J. Korn, Department of Physiology and Neurobiology, Box U-156, University of Connecticut, Storrs, CT 06269, USA. E-mail: korn@oracle.pnb.uconn.edu
S. R. Ikeda, Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912, USA.

tance of Na^+ was 7.87 ± 0.22% (n = 3) of that of K^+ at +20 mV (Fig. 3B).

The data are consistent with a model of selectivity for Kv2.1 that is similar to that proposed for L-type Ca^{2+} channels. This model, in which ions compete for occupancy of the conduction pathway, predicts that as the higher affinity ion is added in relatively low concentrations, macroscopic currents through the channels should reflect permeation of both ions. An alternative possibility is that low concentrations of the high-affinity ion would completely block conduction of the lower affinity ion without itself conducting. For example, at low concentrations, the high-affinity ion may occupy the pore and exclude the low-affinity ion but may lack the energy required to traverse the channel. It has been impossible to test these alternatives with Ca^{2+} channels because the micromolar concentrations of Ca^{2+} required to block Na^+ conductance completely are too low to generate a measurable Ca^{2+} current. These two possibilities can, however, be distinguished for permeation of K^+ and Na^+ through Kv2.1, because millimolar concentrations of K^+ are required to inhibit Na^+ conductance.

If permeation of K^+ and of Na^+ through Kv2.1 are mutually exclusive, mixtures of the two ions should produce currents that reverse through one or the other equilibrium potential. At some concentration between 3 and 10 mM, the reversal potential should switch from the Na^+ equilibrium potential to the K^+ equilibrium potential. In contrast, if both K^+ and Na^+ can permeate Kv2.1 at low concentrations of K^+, mixtures of K^+ and Na^+ should result in an intermediate reversal potential, governed by the permeability ratio, P_K/P_{Na}, of the two ions.

With equimolar Na^+ inside and out, extracellular addition of 3 or 10 mM K^+ shifted the reversal potential by 7.3 ± 0.4 mV (mean ± SEM; n = 4) and 30.7 ± 1.9 mV (n = 4), respectively (Fig. 4). Both step currents and tail currents shifted similarly. The P_K/P_{Na} ratio was higher at 10 mM K^+ than at 3 mM K^+ (32.7 ± 3.7 versus 15.5 ± 1.1), which is consistent with the competition mechanism in a multioccupancy channel (*2*, *12*).

The mechanism of selectivity for K^+ over Na^+ is qualitatively well described by a model in which K^+ and Na^+ compete for occupancy of the pore, with K^+ having a higher affinity than Na^+ for at least one intrapore binding site. Despite the higher affinity of K^+ for the channel, K^+ permeation produced a larger current, which is consistent with a model in which electrostatic repulsion contributes to the K^+ conductance (*2*, *6*, *13*).

The only channel previously demonstrated to select among physiologically relevant ions by a competition mechanism is the L-type, voltage-gated Ca^{2+} channel (*6*). Our data demonstrate that a similar mechanism operates in a delayed rectifier K^+ channel cloned from human brain cortex (Kv2.1). It is likely that this mechanism also operates in at least two other delayed rectifier K^+ channels in cells from two other animal species, rat superior cervical ganglion neurons and chick dorsal root ganglion neurons (*7*). The fact that this competition mechanism occurs outside of the Ca^{2+} channel domain suggests that it does not require special properties associated with Ca^{2+} binding sites (*6*).

Kv1.5 also allowed a small but measurable Na^+ permeation in the absence of K^+ (*14*). Although it is possible that the mechanisms by which Kv2.1 and Kv1.5 select for K^+ over Na^+ differ, the structural similarity of the two channels suggests that the difference in their abilities to conduct Na^+ is quantitative in nature. The simplest possibility is that a specific combination of amino acid residues within the pore forms binding sites with

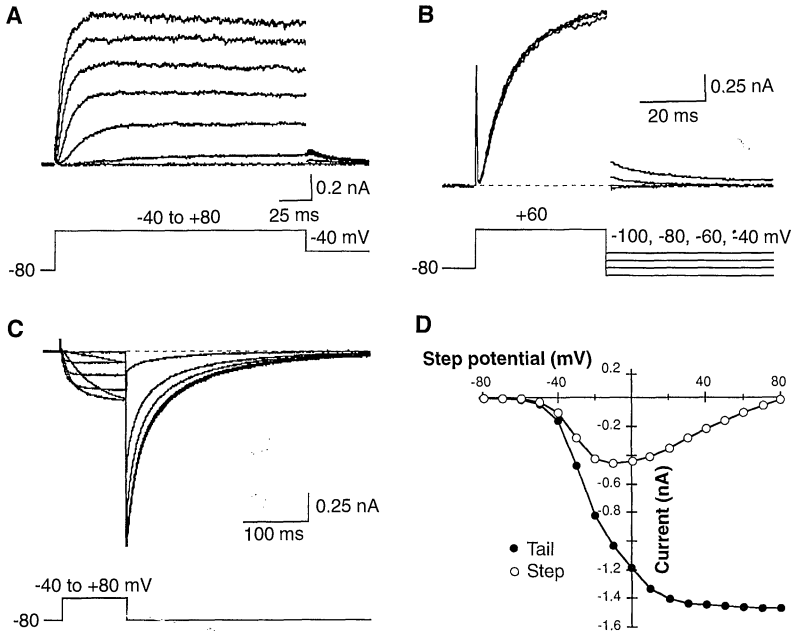

Fig. 1. Effect of K^+ removal on Na^+ permeation through Kv2.1. (**A**) K^+ currents were evoked by 200-ms depolarizations to voltages between −40 and +80 mV. Repolarization was to −40 mV to reveal the outward tail current. Solutions for this and other figures are as described (*10*). (**B**) K^+ currents were evoked by a depolarization to +60 mV. Repolarization to different potentials revealed the current reversal potential of −80 mV. (**C**) Currents recorded in the absence of K^+ were evoked by 100-ms depolarizations to voltages between −40 and +80 mV. (**D**) Current-voltage curves showing the Na^+ current magnitude during the depolarizing step (○) and repolarization (●) (measured 5 ms after repolarization) as a function of step potential. Measurements were taken from the cell in (C).

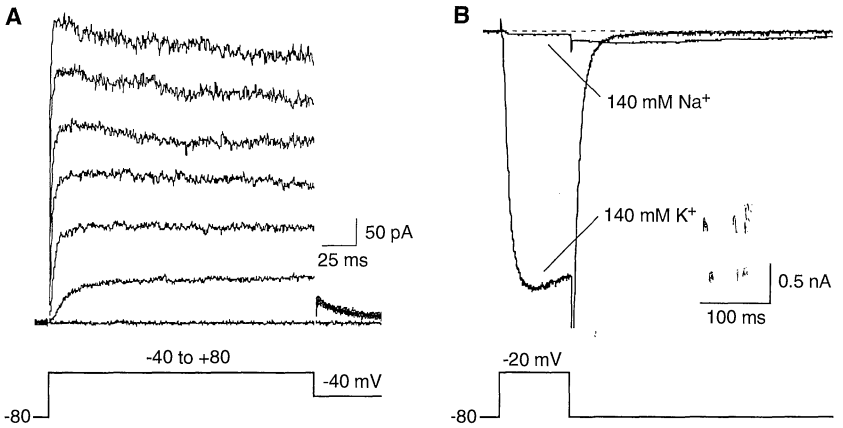

Fig. 2. Effect of K^+ removal on Na^+ conductance in Kv1.5. (**A**) K^+ currents as described for Fig. 1A. (**B**) Currents evoked by depolarizations to −20 mV, measured in a single cell that contained Kv1.5, in the presence of 140 mM Na^+ (and 0 mM K^+) and in the presence of 140 mM K^+ (and 0 mM Na^+). Tail current in 140 mM K^+ is cut off. Solutions were as described (*10*), with the exception of the equimolar substitution of external Na^+ (140 mM Na^+) by K^+ (140 mM K^+).

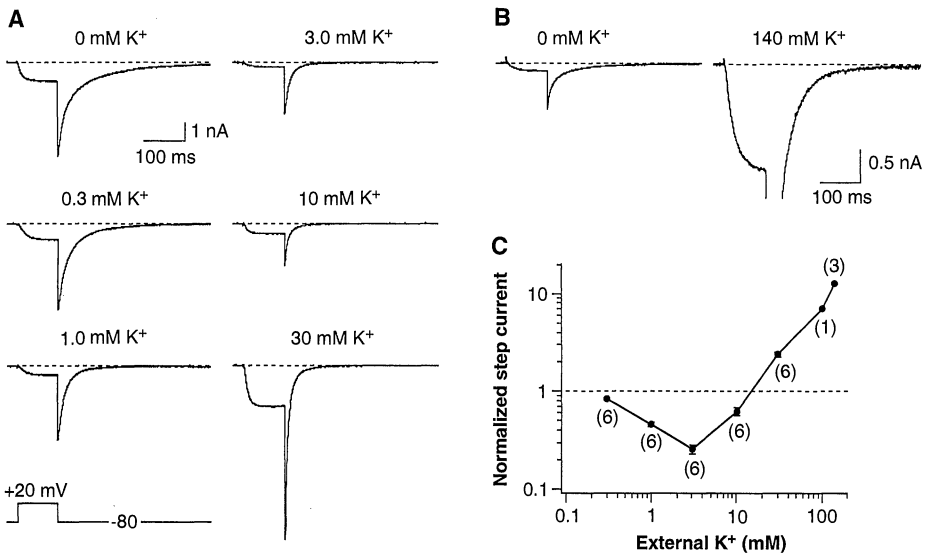

Fig. 3. Anomalous mole fraction behavior between K^+ and Na^+ in Kv2.1. Inward currents were evoked by 100-ms depolarizations to +20 mV. Repolarization was to −80 mV. (**A**) Currents in the presence of various extracellular K^+ concentrations. For currents at 0 to 3 mM K^+, the extracellular Na^+ concentration was 140 mM. At 10 and 30 mM K^+, K^+ was substituted for equimolar concentrations of Na^+. (**B**) Inward currents from a different cell in 140 mM Na^+ (0 mM K^+) and 140 mM K^+ (0 mM Na^+) external solutions. (**C**) Inward current magnitude during the voltage step to +20 mV as a function of K^+ concentration. Currents were normalized to that evoked in the presence of 140 mM Na^+ and 0 mM K^+ (dashed line). Values are means ± SEM; numbers in parentheses represent the number of cells tested.

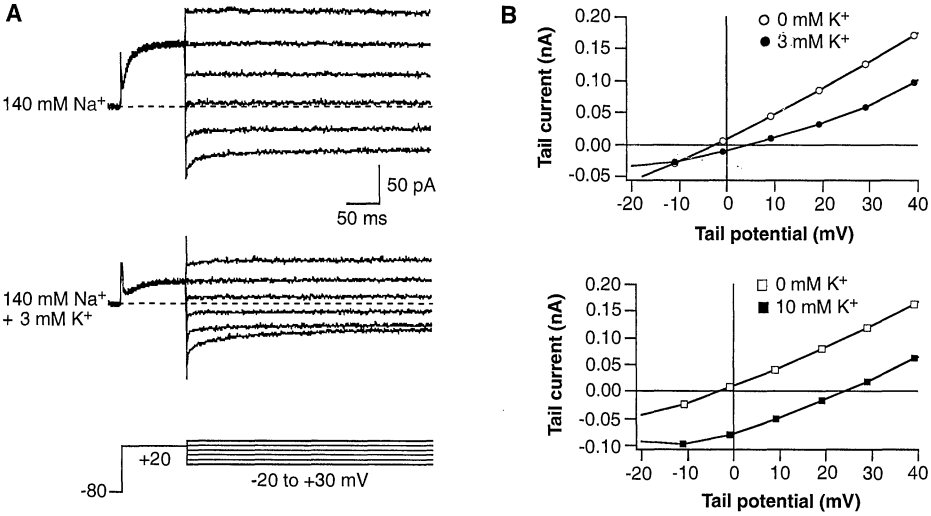

Fig. 4. Reversal potential shifts for Kv2.1 in mixtures of K^+ and Na^+. (**A**) Currents recorded in the presence of equimolar (140 mM) internal and external Na^+ plus 0 or 3 mM K^+. A depolarizing voltage step to +20 mV was followed by repolarization to different potentials that ranged from −20 to +30 mV. (**B**) Tail current-voltage curves (measured 5 ms after repolarization) showing the shift in reversal potential produced by addition of 3 mM (top) or 10 mM (bottom) extracellular K^+.

different affinities for different ions (*15*). However, it appears that channel occupancy by permeant ions markedly alters the three-dimensional structure of the pore (*16*). Consequently, the formation of an intrapore binding site may in part depend on a conformational change in the channel, produced by an ion occupying the permeation pathway. Differences in the ability to conduct Na^+ may thus result from differences in the flexibility of the two channels, which may involve structural determinants outside the permeation pathway. Molecular studies of Kv2.1 and Kv1.5 should help to clarify these issues.

REFERENCES AND NOTES

1. A. L. Hodgkin and R. D. Keynes, *J. Physiol. (London)* **128**, 61 (1955).
2. B. Hille and W. Schwarz, *J. Gen. Physiol.* **72**, 409 (1978).
3. F. Bezanilla and C. M. Armstrong, *ibid.* **60**, 588 (1972).
4. G. Yellen, *ibid.* **84**, 157 (1984).
5. B. Hille, *ibid.* **61**, 669 (1973); H. Reuter and C. F. Stevens, *J. Membr. Biol.* **57**, 103 (1980); A. L. Blatz and K. L. Magleby, *J. Gen. Physiol. (London)* **84**, 1 (1984); L. Heginbotham and R. MacKinnon, *Biophys. J.* **65**, 2089 (1993).
6. P. Hess and R. W. Tsien, *Nature* **309**, 453 (1984); W. Almers and E. W. McCleskey, *J. Physiol. (London)* **353**, 585 (1984).
7. Y. Zhu and S. R. Ikeda, *J. Physiol. (London)* **468**, 441 (1993); M. J. Callahan and S. J. Korn, *J. Gen. Physiol.* **104**, 747 (1994).
8. Mouse L cells (a fibroblast cell line) were obtained from the American Type Culture Collection (Rockville, MD) and maintained as described (*16*). These cells contain no K^+, Ca^{2+}, or Na^+ channels that are activated by voltage between −100 and +100 mV. Complementary RNA was prepared as described (*16*) [S. R. Ikeda, F. Solar, R. D. Zuhike, R. Joho, D. L. Lewis, *Pfluegers Arch.* **422**, 201 (1992)]. Cells were injected with RNA, and injected cells were identified as described (*16*). Currents were recorded 14 to 24 hours after injection.
9. O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pfluegers Arch.* **381**, 85 (1981). Currents were recorded at room temperature (21° to 24°C) and recording pipettes were prepared from N51A glass.
10. In Figs. 1A, 1B, and 2A, the external solution contained 5.4 mM KCl, 140 mM NaCl, 2 mM $CaCl_2$, 0.8 mM $MgCl_2$, 10 mM Hepes-NaOH (pH 7.4), and 15 mM glucose (osmolality, 295 mosmol kg^{-1}). The pipette solution contained 125 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA, 10 mM Hepes-KOH (pH 7.2), and 4 mM adenosine triphosphate (Mg^{2+} salt) (osmolality, 270 mosmol kg^{-1}). In Figs. 1C and 2B and in most other experiments, the external solution contained 135 mM NaCl, 2 mM $CaCl_2$, 10 mM Hepes-NaOH (pH 7.4), and 45 mM sucrose (osmolality, 340 mosmol kg^{-1}), and the pipette solution contained 155 mM *N*-methylglucamine (NMG^+), 1 mM $CaCl_2$, 11 mM EGTA, [14 mM creatine phosphate (pH 7.2, tris), 4 mM adenosine triphosphate (Mg^{2+} salt), 0.3 mM guanosine triphosphate (Na^+ salt)], 20 mM HCl, 10 mM Hepes-methanesulfonic acid (pH 7.2) (osmolality, 303 mosmol kg^{-1}); substitutions are detailed in figure legends. The pipette solution for experiments in Fig. 4 contained 140 mM NaCl instead of NMG^+.
11. The instantaneous current magnitude at −80 mV reached a minimum at 10 mM K^+.
12. Permeability ratios were calculated from the Goldman-Hodgkin-Katz equation.
13. D. G. Levitt, *Biophys. J.* **22**, 209 (1978).
14. We cannot rule out the possibility that incomplete dialysis of the cells left some residual K^+, which might be enough to prevent a more significant Na^+ permeation. This residual concentration would have to be less than ~20 μM [S. J. Korn and S. R. Ikeda, unpublished observations).
15. A recent study suggested that a highly conserved, eight–amino acid signature sequence is an important contributor to the selectivity mechanism in K^+ channels [L. Heginbotham, Z. Lu, T. Abramson, R. MacKinnon, *Biophys. J.* **66**, 1061 (1994)]. All mutations in this sequence that permitted Na^+ to permeate also permitted Li^+ to permeate. In contrast, removal of K^+ permitted the chick dorsal root ganglion delayed rectifier (and perhaps also Kv2.1) to conduct Na^+ but not Li^+ (*7*). These observations suggest that the structural components that confer selectivity may not reside entirely in the signature sequence.
16. S. R. Ikeda and S. J. Korn, *J. Physiol. (London)* **486**, 267 (1995).

17. We thank R. Joho for supplying the human Kv2.1 and Kv1.5 complementary DNA clones; Y. Zhu for performing preliminary experiments; E. Zboran for technical assistance; and L. Heginbotham, R. Horn, J. LoTurco, and A. Moiseff for discussions and comments on earlier versions of the manuscript. Supported in part by the University of Connecticut Research Foundation (S.J.K.) and the American Heart Association–Georgia Affiliate (S.R.I.).

14 February 1995; accepted 5 June 1995